PROTEIN EXPRESSION FROM MULTIPLE NUCLEIC ACIDS

This application is the National Stage of International Application No. PCT/EP2008/008523, filed Oct. 9, 2008, which claims the benefit of EP 07019999.7 filed Oct. 12, 2007, which is hereby incorporated by reference in its entirety.

The current invention is in the field of polypeptide production. More precisely it is reported the production of an immunoglobulin in a mammalian cell whereby the mammalian cell is transfected with different vectors each comprising an expression cassette for the immunoglobulin of interest.

BACKGROUND OF THE INVENTION

Expression systems for the production of recombinant polypeptides are well-known in the state of the art and are described by, e.g., Marino, M. H., Biopharm. 2 (1989) 18-33; Goeddel, D. V., et al., Methods Enzymol. 185 (1990) 3-7; Wurm, F., and Bernard, A., Curr. Opin. Biotechnol. 10 (1999) 156-159. Polypeptides for use in pharmaceutical applications are preferably produced in mammalian cells such as CHO cells, NS0 cells, SP2/0 cells, COS cells, HEK cells, BHK cells, PER.C6® cells, or the like. The essential elements of an expression plasmid are a prokaryotic plasmid propagation unit, for example for *E. coli*, comprising a prokaryotic origin of replication and a prokaryotic selection marker, an eukaryotic selection marker, and one or more expression cassettes for the expression of the structural gene(s) of interest each comprising a promoter, a structural gene, and a transcription terminator including a polyadenylation signal. For transient expression in mammalian cells a mammalian origin of replication, such as the SV40 Ori or OriP, can be included. As promoter a constitutive or inducible promoter can be selected. For optimized transcription a Kozak sequence may be included in the 5' untranslated region. For mRNA processing, in particular mRNA splicing and transcription termination, mRNA splicing signals, depending on the organization of the structural gene (exon/intron organization), may be included as well as a polyadenylation signal.

Expression of a gene is performed either as transient or as permanent expression. The polypeptide(s) of interest are in general secreted polypeptides and therefore contain an N-terminal extension (also known as the signal sequence) which is necessary for the transport/secretion of the polypeptide through the cell into the extracellular medium. In general, the signal sequence can be derived from any gene encoding a secreted polypeptide. If a heterologous signal sequence is used, it preferably is one that is recognized and processed (i.e. cleaved by a signal peptidase) by the host cell. For secretion in yeast for example the native signal sequence of a heterologous gene to be expressed may be substituted by a homologous yeast signal sequence derived from a secreted gene, such as the yeast invertase signal sequence, alpha-factor leader (including *Saccharomyces, Kluyveromyces, Pichia*, and *Hansenula* α-factor leaders, the second described in U.S. Pat. No. 5,010,182), acid phosphatase signal sequence, or the *C. albicans* glucoamylase signal sequence (EP 0 362 179). In mammalian cell expression the native signal sequence of the protein of interest is satisfactory, although other mammalian signal sequences may be suitable, such as signal sequences from secreted polypeptides of the same or related species, e.g. for immunoglobulins from human or murine origin, as well as viral secretory signal sequences, for example, the herpes simplex glycoprotein D signal sequence. The DNA fragment

encoding for such a presegment is ligated in frame to the DNA fragment encoding a polypeptide of interest.

Today CHO cells are widely used for the expression of pharmaceutical polypeptides, either at small scale in the laboratory or at large scale in production processes. Due to their wide distribution and use the characteristic properties and the genetic background of CHO cells is well known. Therefore, CHO cells are approved by regulatory authorities for the production of therapeutic proteins for application to human beings.

In EP 0 569 678 are reported double transfectants of MHC genes as cellular vaccines for immunoprevention of tumor metastasis. WO 97/08342 reports an improved method for measuring the activity of a promoter sequence in a mammalian cell using a reporter gene. The use of anti-RhoA and anti-RhoC siRNAs in order to inhibit specifically RhoA or RhoC synthesis is reported in WO 2005/113770. A method for the recombinant production or expression of eukaryotic alkaline phosphatase mutant in yeast cells is reported in U.S. Pat. No. 7,202,072. WO 2001/038557 reports a method of screening multiply transformed cells using bicistronic expression of fluorescent proteins. A method for producing recombinant eukaryotic cell lines expressing multiple proteins or RNAs of interest is reported in WO 1999/47647. Systems, including methods, compositions, and kits, for transfection of cells with transfection materials using coded carriers are reported in WO 2003/076588. In U.S. Pat. No. 5,089,397 is reported an expression system for recombinant production of a desired protein comprising CHO cells transformed with a DNA sequence having the desired protein coding sequence under the control of the human metallothionein-II promoter. A method for producing recombinant proteins is reported in US 2003/0040047. Lamango et al. (Lamango, N. S., et al., Arch. Biochem. Biophys. 330 (1996) 238-250) report the dependency of the production of prohormone convertase 2 from the presence of the neuroendocrine polypeptide 7B2. The transfection of a BPV-1-based expression vector into cells harboring unintegrated replicating BPV-1 genomes is reported by Waldenstroem, M., et al., Gene 120 (1992) 175-181. U.S. Pat. No. 4,912,038 reports methods and vectors for obtaining canine and human 32K alveolar surfactant protein. In WO 89/10959 are reported recombinant DNA techniques and the expression of mammalian polypeptides in genetically engineered eukaryotic cells. A repeated co-transfer of an expression vector for human growth hormone and an expression vector for a selection marker gene is reported in DD 287531.

SUMMARY OF THE INVENTION

A first aspect of the current invention is a method for the recombinant production of a heterologous immunoglobulin which is secreted to the cultivation medium in a CHO cell comprising:

a) providing a CHO cell, which is
   adapted to growth in suspension culture,
   adapted to growth in serum-free medium,
   mycoplasm free, and
   optional virus free,
b) providing a nucleic acid comprising
   a prokaryotic origin of replication,
   a first nucleic acid sequence conferring resistance to a prokaryotic selection agent,
   a second nucleic acid sequence encoding the heavy chain of said heterologous immunoglobulin, and/or a third nucleic acid sequence encoding the light chain of said heterologous immunoglobulin,